Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.4, No.9, 2014
www.iiste.org

Effect of Seed Cluster and Gibberelic Acid (GA_3) on the Gender Development of *Salacca edulis* REINW. (Snake fruit) Grown on BRIS soil

Razifah MR[1*], Mamat AS[2], Adzemi MA[1], Nor Shariah S[1]
1. School of Science and Food Technology, Universiti Malaysia Terengganu
21030 Kuala Terengganu, Malaysia.
2. School of Bioprocess Engineering, Universiti Malaysia Perlis, 02500 Kangar, Perlis, Malaysia
* E-mail of corresponding author: razifah@umt.edu.my

Abstract
Salacca edulis REINW. (Snake fruit) is a monocotyledon plant belonging to the palmae family. Climate requirement for this plant is equatorial. A series of experiments on the growth and development of *S. edulis* were conducted in the greenhouse of University Malaysia Terengganu (UMT) and in the field on undeveloped agricultural land (BRIS soil) in Tumpat, Kelantan. A nursery study was undertaken for six months followed by a field study for 24 months. The aim of both studies was to determine the possible effect of GA_3 and seed clusters in the fruit of *S. edulis* on gender determination of the plant during early growth. Gender determination is a major problem in *S. edulis* orchard industry where farmers often fail to identify male and female plants at the earlier stage because of their dioecious nature and morphological similarity, except the flower which appears during the reproductive stage. Based on the results of this study, there were strong relationships between the number of seeds per fruit and plant gender. For female plants; single seed produced 20%, two seeds produced 33% and three seeds produced 42%. Results showed a positive correlation between number of seeds and percentage of female plants they produced. It was concluded that fruit containing three seeds gave a better combination of male and female *S. edulis* planting materials and it was good for commercial planting. Application of GA_3 treatment did not give a strong effect for gender determination. However, the highest rate of GA_3 (100 ppm) promoted early flowering. The male flowers contain auxin: Indole-acetic-acid (IAA) and Abscisic acid (ABA) but have no gibberellins (GA_3). Meanwhile, the female flowers contain IAA and GA but have no ABA. This finding also proves that, with proper management such as the use of right organic fertilizer and irrigation system, *S. edulis* can be planted on BRIS soil which has poor physical and chemical properties due to its lack of clay.
Keywords: Seed cluster, BRIS soil, **g**ibberelic acid, *Salacca edulis,* dioecious

Introduction

Fruits are an important part of the human diet contributing essential energy, vitamin, minerals and fibers. The genus of *Salacca* has about 20 species found through a broad region of Southeast Asia. The classification of this species is made based on the fruit characteristics such as size, shape, skin color and flesh quality especially taste, crispiness and aroma (Ringgot and Voon, 1992). It is belongs to the family Palmae and has important economic value because of their non-seasonal behavior. Among the *Salacca* species, *Salacca edulis* REINW. has a high potential as an export crop for the fresh market. There's worldwide market and demand for new subtropical and tropical fruits with a high nutritional and sensory value. *S. edulis* is an exotic plant and was only recently introduced into Malaysia and future demand of the fruit is expecting to rise yearly (Mamat *et al.,* 1996).

S. edulis grows well mostly in areas 500-700 m above sea level with the climate types of A, B, or C based on Schmidt-Fergusson classification (Sudaryono, 1992). Traditionally, *S. edulis* is planted on mineral soil but BRIS soil is also suitable because of their root system. Audesirk *et al.,* (2007) reported that *S. edulis* has a fibrous root system because they are monocotyledon. Terraced land is the best to grow these *S. edulis* plantations. This is the first trial to grow *S. edulis* on BRIS soil. Soils of beach ridges interspersed swales (BRIS) dominate large parts of the coastal areas of the east cost of Peninsular Malaysia (Rasidah *et al.,* 2010). The main factors limiting this soil for agriculture are their poor soil structure, high percentage sand, low water holding capacity, low organic matter content, high surface temperature, lack of soil fauna, and low microbial activities (Abdul Wahab and Osman, 1989). During the dry period, the high air and surface soil temperatures can cause leaf scorching and wilting of the plant (Amir, 1999). The use of organic fertilizer is suggested for BRIS soil to improve their water holding capacity and reduce environmental pollution from agricultural waste. With proper soil management, BRIS soil is suitable for roselle, cashew, water melon and vegetables such as chilli, cabbage, maize, sweet corn and sweet potato. This present study also investigates the growth performance of *S. edulis* planted on BRIS soil. This study may open a new window of opportunity in the use of BRIS soil for commercial production of crop species normally planted on other soils.

The strategy to improve production and productivity of the fruits should focus on the availability of planting

materials. *S. edulis* is widely cultivated throughout Malaysia and is generally grown from seed. The oldest, easiest and most common type of plant propagation is through the use of seeds (Hartmann *et al.,* 1997). The uses of seed as planting material give rise to variation in pant sexes because *S. edulis* is dioecious. Propagation using quartering technique, rhizome and sucker (Hashim, 1993) do exist but these are slow and the mortality rates are high and cannot be practiced for large scale production. Tissue culture was not success for this species not like in *Elaeis guineenis* (oil palm). From an economic point of view, growing *S. edulis* from non-selected seeds cannot maximize profit. All plant produced for this species are either male or female. The recommended gender ratio for commercial planting is 1 (male): 10 (female) because of ability to give enough pollinator plants (Sumeru, 2002). In nature, a bunch of *S.edulis* fruits consisted of 90% fruit with a three seeds cluster, 9% with a two seeds cluster and 1% with a one seed cluster (Razifah *et al.,* 2007). Therefore, the study on the effect of seed clusters of *S. edulis* for gender determination is important for commercial planting.
The seeds of *S. edulis* are recalcitrant. The seed generally survive from two to four weeks at ambient temperature. Due to the slow and irregular germination of palm seeds, there has been a great deal of interest in any replanting treatments that might speed germination or that could result in more even rates of germination percent of arecacea palms (Rauch *et al.,* 1982). Growth regulators are used to protect plants from a variety of stresses. Advantages of seed treatments with growth regulators include less usage and drift of the product, and simplicity of application (Fletcher *et al.,* 2000). The effects of gibberellic acid (GA_3) on gender expression were also studied. The endogenous hormones in *S. edulis* flowers such as abscisic acid (ABA), GA_3 and auxin were also analyzed.

Materials and methods

Location of the study

The nursery study was contacted at University Malaysia Terengganu (UMT) and the field study was conducted at Kg. Pengkalan Nangka, Tumpat, Kelantan, Malaysia.

Nursery operation

2.2.1 Seed selection

Mature fruit of *S. edulis* were obtained from a commercial farm in Jeli, Kelantan (Figure 1). Seeds were manually extracted from fruits and were classified according to their morphological characteristic. *S. edulis* seeds were graded into three classes based on type of seed clusters; one, two and three seeds per fruit. Each seed has their own tagging.

2.2.2 Seed germination

After the classification, seeds were immersed in water for 24 hours (at room temperature) to speed up the germination. Meerow (2006) and Durrawati (1994) reported that most palm seeds required a soak in water to soften (ferment) the seed. Once fermented, the seeds were ready to be soaked in GA_3 solution that ranged from 0 to 100 ppm (0, 10, 50 and 100 ppm). Seeds were transferred to wet tissue with an occasional light application and maintained at room temperature for germination. Seeds were checked for germination every three days. Germination was deemed to have occurred when the cotyledon emerged from the seed coat. After two weeks, the seeds which showed a potential for growth were selected. The seeds having coleoptiles between 2-5 cm were chosen and ready for planting into polybags. Prior to planting into polybags, seeds were soaked again in gibberellic acid (GA_3) at the same concentration. The seedlings were then left for six months in the greenhouse before they were transplanted into the field. The same tagging was used for nursery and field study, respectively.

Field activities

The field study was conducted on BRIS soil. Traditionally, *S. edulis* is planted on mineral soil. This is the first trial to grow *S. edulis* on BRIS soil that are synonymous with sandy soil. The seedlings were planted in holes (90 cm x 90 cm x 60 cm) filled with top soil, charcoal, chicken dung and an empty bunch of palm oil. All the plants were applied with 25 mL of GA_3 (0, 10, 50 and 100 ppm) one week after transplanting. GA_3 treatments were sprayed in aqueous solution onto the leaves. This method follows Mukherjee (1969) with minor modification. Treatments were applied early in the morning and plants were kept well watered using drip irrigation. As the source of the major nutrients required by the plants, 100 g of organic fertilizer (HUMIFLEX® 10:10:10) were applied one month after transplanting and also every two weeks for maintained the nutrient requirement for plant growth.

2.*4* ***Plant hormone analysis***

2.4.1 Standard and chemicals

Standards of auxin [Indole-3-Acetic Acid (IAA)] and abcisic acid (ABA) and gibberelic acid (GA_3) were obtained from Sigma. Solvent such as methanol and acetone were obtained from Merck.

2.4.2 Sampling and extraction for plant hormone analysis

The male and female flowers were picked from the mother plant of *S. edulis.* The flowers were cut few mm below the calyxes', and the pedicels were wrapped in aluminium foil to minimize the loss of water. Plant material (10-20 g) was ground in methanol. In the laboratory, the extract was transferred into a flask with fresh methanol and the volume was adjusted to 20 mL for each g of fresh weight samples. The extract was allowed to stand for 24 hours at 0°C after addition of internal standards and was then vacuum-filtered through Whatman No 42 paper.

2.4.3 HPLC analysis

Analysis by High Performance Liquid Chromatography (HPLC) was carried out on an Agilent 1100 HPLC (Agilent Technologies, Palo Alto, CA) equipped with DAD 1100 diode array detector. The methanolic extract was filtered through a 0.45 µM and was passed through a Zorbax C_{18} column. The mobile phases were methanol and 0.67 % acetic acid (pH 3.0). Separation of IAA, ABA and GA was archived using a concave gradient of 80:20 to 60: 40 methanols/ 0.67 acetic acid (pH 3.0) in a Zorbax C18 column (4.6 mm x 250 mm, pore size 5 Mm0, fitted with a sentry guard column. The injection volume was 10 µL and the run time was 10 minutes. Detection wavelength used in this study was 220 nm with 1.0 $mL.min^{-1}$ flow rate.

2.4.4 Statistical analysis

Data obtained for each parameter were subjected to statistical analysis using SPSS Version 13.0. Repeated measures by analysis of variance procedure were used only on parameters that involved repetition over time.

Results and discussion

The effect of GA_3 application on plant growth and the relationship between type of seed clusters and the gender development of *S. edulis* were studied. Table 1 showed the effect of GA_3 on the plant height of *S. edulis.* It shows that GA_3 treatment does not significantly affect for plant height at all growth phases. Since there was no significant difference in plant height between the GA_3 concentrations, a greater range may be needed to find an optimum concentration of GA_3. Using a greater range of GA_3 concentrations at a lower concentration may show that less GA_3 is needed to obtain equal or greater plant height.

However, it must be considered that BRIS soil is inherently poor in nutrient reserves and subjected to a remarkable loss of available nutrient due to a low degree of surface soil aggregation resulting from high level of the sand fraction (more than 92%). *S. edulis* was grown successfully on BRIS soil under controlled conditions. This study has proved that BRIS soil can be used to plant *S. edulis* for commercial uses.

Table 1: The effect of GA_3 on the plant height of *Salacca edulis*

Treatment	**Plant height (cm)**			
	6 months	**12 months**	**18 months**	**24 months**
0	34.86a	71.64a	96.69a	166.14a
10	34.21a	69.77a	91.54a	166.61a
50	35.69a	71.11a	93.72a	160.67a
100	35.39a	71.50a	88.37a	163.42a

*Value of the same column which contained same letter are not significantly different at the level $p<0.05$ (DNMRT)

The oldest, easiest and most common type of plant propagation is through the use of seeds (Hartmann *et al.,* 1997). Since *S. edulis* is dioecious, seed selection is very practical for commercial planting. The biggest drawback about propagation by seeds is that there is no certainty of whether the plants will be male or female until they start to produce inflorescence, usually around 18 months after field planting (Ringot and Voon, 1994). In this trial, the first flower emerged 23 months after sowing which compares to Sumeru (2002) who reported 23-24 months. After 30 months, the percentage of flowering plants ranged from 50-84 %. Usually, a flower in a male plant appears earlier than in a female plant because of the morphology of the male plant. The plant sexes should be known at planting, to allow optimal spacing of fruit-bearing plants in an orchard and to include enough pollinator plants from the start (Sumeru, 2002).

S. edulis fruits usually have one, two and three seeds cluster per fruit (Figure 2). Type of seed cluster was determined by the number of seeds per fruit which comprised one, two and three seeds per fruit. Sudaryono (1992) reported two ways to differentiate sexes of *S. edulis* seeds for planting is cluster from number of seeds per fruit and seed shape. Ringot and Vood (1994) reported that propagation by *S. edulis* seeds often results in high proportion of male plants (as high as 80 % males).

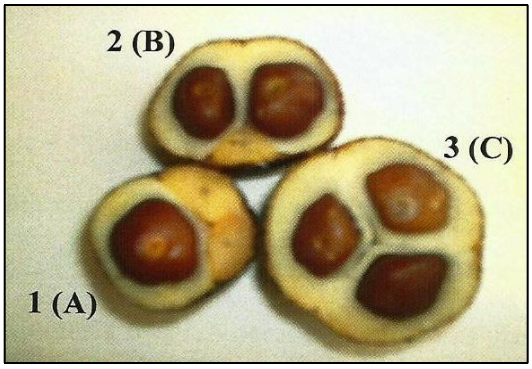

Figure 2: Type of Seed Clusters of *Salacca edulis* REINW.

In this study, the type of seed cluster gave interesting results (Table 2). Data are presented in percentage for number of male and female plants that are related to seed cluster. Data were analysed using cross tabulation tests to investigate their relationship between sexes of the plant and seed cluster. Seeds with **one seed cluster (A)** present 80.5% male and 19.6% female plants. Fruit with **two seed clusters (B)** gave 66.7% male and 33.3% female plants. Meanwhile, **three seed clusters (C)** gave 58% male and 42% female. These results gave supporting evidence that was earlier report by Sudaryono (1992) who stated that fruit with one seed usually gave male plant, whereas fruit with two seeds gave male and female plant but that report was not based on statistical data.

Table 2: Percentage of male and female flowers as expected by type of seed clusters

Type of Seed Cluster	**Flowers clusters (%)**	
	Male	**Female**
1 (A)	80.5	19.5
2 (B)	66.7	33.3
3 (C)	58	42

As shown in Table 3, the results found that different types of seed clusters should be used as a preliminary gender determination. T-value showed that both fruit with one and two seeds gave a high number of male plants. Gender determination from fruit with three seeds did not give significantly different at P=0.05 and 0.01 for male and female plants. In contrast, fruit with three seeds cluster was suggested as planting material. In contrast, Sudaryono (1992) recommended fruit with two seeds for planting.

Table 3: Difference in the type of seed clusters by gender

Seed Clusters	**Male (%)**	**Female (%)**	**t-value**
1	80.5	19.5	5.82**
2	66.7	33.3	2.17*
3	58.0	42.0	1.37

*, ** indicates significant level at P=0.05, 0.01 respectively

Table 4 showed the correlation between the types of seed and gender determination (male). Fruit with one and two seeds were significantly correlated to gender determination, while fruit with three seeds (one seed; p=0.01. two seeds; p=0.05) was not. These results suggest that type of seed positively affects the sexes of plant. It is strongly suggested that fruit with three seeds is most suitable for planting.

Table 4: Correlations between the type of seed clusters and sexes (male)

Seed Clusters	**Sexes**	**Seed 1**	**Seed 2**	**Seed 3**
	1	-0.78**	-0.42*	-0.28
Seed 1	-0.78**	1	0.45*	0.29
Seed 2	-0.42*	0.45*	1	0.23
Seed 3	-0.28	0.29	0.23	1

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2 tailed)

The next study was to test if gender development could be affected by plant hormone. A randomised block design with three replications and the four treatments GA_3; control (0 ppm), 10 ppm, 50 ppm and 100 ppm were used. Table 5 illustrates that GA_3 treatment did not give a strong effect for gender determination. 50 ppm GA_3 treatment gave the highest numbers of female plants compared to any other concentration. Under natural conditions, GA_3 does not exceed more than 50 ppm in plants. At higher levels, it may inhibit the function of GA_3 because at concentration it will causes hormonal imbalance. Applications of GA_3 showed that GA_3 confer less effect for gender determination. Yin (1994) reported that determination of sex in a fundamental biological problem and its understanding has tremendous practical significance, such as in fruit production of many agricultural and horticultural crops.

Table 5: Percentages of male and female flowers of *S. edulis* as affected by GA_3

Treatment (ppm)	Flowers clusters (%)	
	Male	Female
0	72.77a	27.23a
10	72.87a	27.13a
50	61.53a	38.42a
100	75.37a	24.63a

*Value of the same column which contained same letter are not significantly different at the level $p<0.05$ (DNMRT)

Possibly, to alter sexes for this plant a higher GA_3 treatment has to be used and the research about treatment application ought to be continued. Yin (1994) reported that, GA_3 treatment can promote maleness in all monocious plants and enhance flowering in male plants. However, the characteristic is not found in dioecious plants like *S. edulis.* Based on this result, it is suggested that GA_3 treatment has the potential to alter gender development; however the optimum dosage is yet to be determined.

Table 6 presents the comparison of hormone detection in male and female flowers of *S. edulis*. This study was done to support the data for exogenous application. In this study, ABA was detected in male but not in female plants. Meanwhile, IAA was present in both male and female. GA_3 was detected in the female but not in male flower. ABA and GA_3 are inversely correlated.

The finding of endogenous analysis helps us to understand the hormone pattern in male and female flowers of *S. edulis*. Analysis of plant hormone in female plant is represented in Figure 1 which shows that IAA is a major component. From plant hormone analysis, it can be concluded that there is an endogenous hormone difference between male and female flowers. There is correlation between GA_3 in endogenous and exogenous studies. GA_3 in agreement with Riley (1987) who published a procedure for generating female flowers on a male flower, female flowers were induced with 200 to 300 ppm GA_3 but more than 600 ppm hinders any flowering. This relates to the existence of GA_3 in the female flower but not in the male flower.

Table 6: Comparison of retention time between male and female flowers

Hormone	Retention Time		
	Standard	Male	Female
IAA	2.060	2.158	2.065
ABA	2.287	2.267	ND
GA_3	2.339	ND	2.360

The key finding of the study is that a relationship exists between type of seed cluster and gender development in *S. edulis* plants. The outcome of this study can provide vital information for farmers. Classification accuracy was determined by cross-tabulation between the type of seed cluster and gender development of the plant. Significant correlations were recognised between type of seed clusters and gender development in *S. edulis* plants. Three seeds cluster were suggested for planting material because of better combination of male and female plants.

Conclusions

Based on the results of this study, it can be concluded that gender determination of *S. edulis* can be determined by seed selection. There is a strong relationship between type of seed clusters and gender determination. It also found that three seeds cluster gave a better combination of male and female plants of *S. edulis* and that a fruit with a three seeds cluster is most suitable for commercial planting. Since dioecious plants demand for cross-pollination, results of the study are useful for farmers to identify gender determination from seed selection before planting. Plant hormone analysis results showed the significant study for GA_3 treatment. Endogenous study traced that female flowers contains GA_3 and IAA. However, in male flowers, ABA and IAA were detected. It shows that application of GA_3 treatment at appropriate concentration may help plant to alter their sexes.

References

Abdul Wahab, Osman AB (1989) Utilization of BRIS and Tin Tailing Soils for Food and Fruit Production. *Malaysia Society of Soil Science.* National Seminar on Soil Management for Food and Fruit Crop. 29-29 March 1989.

Amir HMS (1999) Manual on the Reforestation with Selected Fast Growing Forest Species on BRIS soils in Setiu, Terengganu, Peninsular Malaysia. Forest Research Institute Malaysia. Kuala Lumpur.
Auderisk T, Auderisk G, Byers BE (2007) Biology with Physiology Life on Earth. 8th Edition. Pearson Education. 923p
Durrawati B (1994) Kesan Pengawalatur Tumbesaran Giberelin Terhadap Tumbesaran dan Perkembangan Anak Benih Salak (S*alacca edulis* REINW.). Dissertation. Universiti Putra Malaysia, Malaysia, 70p
Fletcher A, Gilleney A, Sankhla N, Davies T (2000) Triazoles as plant growth regulators and stress protections. Hort. Rev. 24: 55-138
Hartmann HT, Kester DE, Davies Jr FT, Geneve RL (1997) Plant Propagation; principles and practices. 6th Prentice-Hall. Upper Saddle River, NJ
Hashim AB (1993) Amalan Kultur Tanaman Salak. Ed: Salleh H, Kamariah M, Norlia Y, A. Jamil Z, Hashim AB, Hing TH. Seminar Penanaman Duku Terengganu, Dokong and Salak. MARDI Serdang, Selangor, Malaysia. Pp35
Mamat AS, Siti Aisyah H, Ramlan MF, Rozita A (1996) Physiological responses of Salak seedlings to light and carbon dioxide (CO_2). Ed: Vijayasegaran S, Fauziah M, Mohamad MS, Tarmizi SA. Proceedings of International Fruits Conference, 2: 71-79.
Meerow AW (2006) Palm Seed Germination. IFAS Cooperative Extension Service Buletin 274. Available: http://flrec.ifas.ufl.edu).
Mukherjee (1969) Studies on Flowering Responses of *Urena lobata.* Plant Physiol. 44: 1749-1750
Rasidah W, Rosazlin A, Rozita A (2010) Growth of tongkat ali (*Eurycoma longifolia*) on a sandy beach ridge soil in Malaysia. 19th World Congress of Soil Science, Soil Solutions for a Changing World. Pp 104-107. Published on DVD.
Razifah MR, Mamat AS, Adzemi MA, Sayed Mohd Zain SH (2007) Morphological and Seed Clusters in Early gender Determination od *Salacca edulis* REINW. The paper was presented at National Horticulture Conference at Puteri Pacific Johor Bharu, Johor, Malaysia, 23-25 March 2007.
Riley JM (1987) Gibberellic acid for fruit set and seed germination. CRFG Journal. 19: 10-12
Ringot R, Voon BH (1994) Salak cultivation in Miri, Sarawak. Ed: Salleh H, Kamariah M, Norlia Y, A. Jamil Z, Hashim AB. Seminar Penanaman Duku Terengganu, Dokong and Salak. MARDI Serdang, Selangor, Malaysia.
Sudaryono T (1992) Budidaya Tanaman salak di Indonesia. Ed: Salleh H, Kamariah M, Norlia Y, A. Jamil Z, Hashim AB. Seminar Penanaman Duku Terengganu, Dokong and Salak. MARDI Serdang, Selangor, Malaysia.pp 51-57
Sumeru A (2002) On the agronomy and botany of Salak (*salacca zalacca)*. Wegeningen University Disertation. [Online] Available: http://edepot.wur.nl/192242 (March 3, 2014)
Yin T (1994) A mechanistic model of one hormone regulation both sexes in flowering plants and its application to sex expression in Buchloe dactyloides and Cucumis sativus. PhD Disertation. University of New Jersey, New Brunswick, New Jercy. 135p